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(54) Fecal occult blood test product with positive and negative controls

Testvorrichtung zum Nachweis von okkultem Blut im Stuhl mit positiver und negativer Kontrolle

Dispsositif pour la détermination de sang occulte dans la selle avec contrôle positif et négatif

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Description

The present invention relates generally to an occult blood test product, of the type to be physically placed into a toilet bowl containing a fecal specimen, for detecting the presence of fecal occult blood in an aqueous solution. More particularly, the present invention relates to an improved, reliable fecal occult blood test product which may be utilized with a minimum of human intervention. The principles of the present invention may be employed in the testing for occult blood, ferritin and myoglobin in various biological fluids.

In general terms, the testing of a fecal specimen for occult blood is based on the well-known principle that blood (more particularly hemoglobin) will function as a catalyst and cause oxygen to be liberated from an oxygen donor, with the liberated oxygen thus causing a color change in a chromogenic substance. As such, the test for fecal occult blood is not only well-known, but numerous oxygen donors, numerous chromogens and numerous donor-chromogen pairs have been suggested in the prior literature. In considering the chromogens and oxygen donors which may be used, it should be appreciated that the fecal occult blood test is frequently referred to as a test for the presence of a substance having a peroxidase-like activity.

In addition, there are several principal styles of fecal occult blood test products which have been marketed or described in the literatures. These include slide products, tape products, wipe products, and throw-in-the-bowl products. Slide products require the patient to retrieve part of the stool specimen and, using a spatula or equivalent device, place part of the specimen on a paper part of the slide which is thereafter submitted to a laboratory where a developing solution is applied to the slide. Tape products are typically utilized by a physician after a rectal examination in which instance the physician smears a stool sample on a thin, narrow tape and then a developing solution is applied to the tape. In both of these types of products, the chromogen is guaiac, and the oxygen donor or developing solution is hydrogen peroxide.

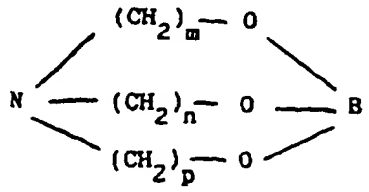
A third type of product is often referred to as the wipe type of product where a form of toilet paper is impregnated with a suitable chemical, typically the chromogen, and after a bowel movement, the patient self-wipes the anal area, and thereafter may apply the developing solution to the paper. As may be appreciated, in each of these types of products there is a need for the patient (or physician) to physically handle or physically contact the fecal specimen. Thus, there is a natural reluctance to employ these types of products, notwithstanding that they are well-known as beneficial screening agents, to assist in the early detection of colorectal cancer and other gastrointestinal disorders.

In November 1979, U.S. Patent No. 4,175,923 issued to William Friend. This patent described a fecal occult blood test product of the throw-in-the-bowl type where a guaiac impregnated sheet was sprayed with a developing solution (hydrogen peroxide) and then placed into a toilet bowl containing a fecal specimen. If blood was present, the blood catalyzed a chromogenic reaction, and a blue color was observed in the toilet bowl. The product described in the Friend patent also included a positive monitor which would turn blue if the chemicals were functioning properly. However, the product as described in the Friend patent still required patient intervention in that the patient was required to apply the developer to the test product.

EP-A-0222700 describes inter alia a throw-in-the-bowl type test product comprising a substantially dry mixture of a 3,3',5,5'-tetramethylbenzidene and an alkali metal borate deposited on a sheet of adsorbent paper sprayed with pressure sensitive adhesive. A second sheet of adsorbent paper is layered along the first. GB-A-2147416 describes a throw-in-bowl test pad having a test area containing a water soluble oxidising agent and a water soluble guaiac substitute.

EP-A-0233144 describes another throw-in-bowl test matrix comprising a substrate containing thereon an oxygen sensitive dye and a peroxygen source capable of reacting with each other in the presence of occult blood; and toilet contaminant monitor testing means consisting essentially of a substrate having an oxygen sensitive dye reactable with strong oxidising agents present as contaminants in solutions.

US-A-4071318 discloses test device for determining the presence of e.g. haemoglobin in a test sample which comprises a carrier matrix incorporated with an indicator capable of producing a detectable response in the presence of hydroperoxide and a peroxidating active substance, and a borate ester having the structure



in which m,n and p are the same or different and are integers of 1 to about 4.

U.S. Patent No. 4,541,987, to Gaudagno issued, September 17, 1985, relates to a throw-in-the-bowl type of product which included both positive and negative test monitors. A product generally in accordance with the teachings of the Guadagno patent has been successfully marketed by Helena Laboratories Corporation, of Beaumont, Texas, under their trademark CS-T™. Helena Laboratories Corporation is the Assignee of the Guadagno patent and Applicant of the present invention. The CS-T™ brand of fecal occult blood test product is commercially successful and medically reliable.

In addition to the CS-T™ brand of fecal occult blood test product, which is in the nature of a test pad or sand-

wich of dry chemicals between layers of paper, a thin film type of product for throw-in-the-bowl fecal occult blood testing is known, see for example US-A-5081040. However, the film type product has not met with success in the market place even though it does not require patient intervention. The film type product does not include self-contained controls or monitors, and the product as marketed has heretofore utilized an external type of positive monitor which must be dropped into the toilet bowl.

Thus, there is a need for a less expensive, reliable, easy to use throw-in-the-bowl type of fecal occult blood test product which is easy to manufacture and provides consistent, reliable results, and which also includes built-in or self-contained positive and negative monitors. These self-contained monitors, of course, aid the patient because the monitors inform the patient if a positive or a negative result should be ignored as being induced by contaminants or being the result of loss of activity by the chromogen or oxygen donor. In this fashion, a patient will know to repeat a test rather than rely upon false test results.

Hence, the present invention responds to these needs by providing an improved formulation of film-type throw-in-the-bowl fecal occult blood type product.

The present invention provides an improved test product for the determination of the presence of occult blood, which product may be placed into a toilet bowl without patient intervention as heretofore described. The test product of the present invention includes not only a specimen test area but, in addition, a positive monitor area and a negative monitor area.

According to the present invention there is provided a test sheet for the determination of the presence of a substance having a peroxidase-like activity such as haemoglobin in an aqueous solution, comprising:

a sheet having a specimen test area and a positive monitor area and a negative monitor area, said sheet comprising an inert water insoluble matrix;
said specimen test area having deposited thereon a first composition comprising at least one oxygen donor reagent dissolved in a solvent comprising a surfactant, at least one chromogen reagent dissolved in a solvent comprising a surfactant, and capable of being oxidized by the oxygen donor in the present of a substance having the peroxidase-like activity, to provide a visually observable change of colour; and
said positive monitor test area having deposited thereon said first composition and a second composition comprising a substance having peroxidase-like activity.

Also according to the present invention there is provided a method of making a test sheet which comprises dissolving said at least one chromogen reagent in a solvent comprising a surfactant to form a first portion of said first composition, separately dissolving said at least one oxygen donor in a solvent comprising a surfactant to form a second portion of said first composition, adding said second portion to said first portion to form said first composition, printing said first composition on a sheet in said specimen test area and said positive monitor area but not in said negative monitor area and printing a second composition comprising a substance having peroxidase-like activity in said positive monitor area.

Further, according to the present invention there is provided a method of making a test kit for the determination of the presence of a substance having peroxidase-like activity comprising the steps of:

providing a sheet having a specimen test area and a positive control area, said sheet comprising an inert water insoluble matrix;
applying a test ink to said specimen test area and said positive control area, said test ink comprising at least one oxygen donor reagent dissolved in a solvent comprising a surfactant, at least one chromogen reagent dissolved in a solvent comprising a surfactant, and capable of being oxidized by the oxygen donor in the presence of a substance having the peroxidase-like activity, to provide a visually observable change of colour; and
applying a positive monitor ink on said test ink in said positive monitor area, said positive monitor ink comprising a substance having the peroxidase-like activity.

The test product is, in a preferred embodiment, a sheet of filter paper upon which the chromogen and oxygen donor are printed as a test ink. Thus, the test ink includes at least one oxygen donor reagent and at least one chromogen reagent capable of undergoing a visible color change when oxygen is liberated by the oxygen donor. The test ink may include a stabilizer for preventing premature interaction between the oxygen donor and the chromogen. The test ink is printed on the test sheet in the specimen test area and in the positive monitor area but not in the negative monitor area. A polymer barrier is then printed over the test ink. Lastly, a positive monitor ink is printed in the positive monitor area, on top of the polymer barrier. The purpose of the positive monitor ink is that if the chemicals are functioning properly, then there will be a visible color change in the positive monitor area. Thus, the positive monitor ink includes a substance which will catalyze the liberation of oxygen from the oxygen donor reagent.

Preferably the test ink includes at least one water soluble polymer for immobilising the chromogen and oxygen donor.

The invention, together with advantages which may be attained by the principles of the present invention, will become more apparent upon reading the following detailed description of the invention in conjunction with the drawings.

In the drawings, the single Figure illustrates the test

product of the present invention.

The single Figure illustrates a test product or test pad 10 in accordance with the present invention. The pad 10 is illustrated as a generally rectangular sheet having a large test area 12, which may advantageously occupy approximately more than half of the surface area of the sheet, and a monitor or control region 14 which may include a positive monitor test area 16 and a negative monitor test area 18. The two monitor test areas 16, 18, are approximately equal to each other in size. Suitable legends or directions 20, 22 and 24 may be included on the test product.

The entire test pad 10, in accordance with the principles of the present invention, may be a sheet of water insoluble matrix or material such as Whatman filter paper having fibers or interstitial spaces. A suitable alternative to the Whatman filter paper would be Schleicher & Schuell No. 596 filter paper. The overall dimensions of the sheet may be 10 x 13 cm. The paper as described is of the short fiber type which is sufficiently water repellant for the desired purpose such that the paper does not prematurely shred when initially immersed into an aqueous solution. It should be pointed out that all dimensions and ratios of the specimen test area and monitor areas are for illustrative purposes and should not be taken as a limitation on the present invention.

A test ink is deposited, i.e., printed on the entire sheet except the negative monitor test area 18. The printing deposits the test ink onto the test sheet, and the terms "printing" and "depositing" are used in the broad sense to include not only placing the ink on the surface of the sheet but also to include any desired degree of impregnation of the ink into the sheet. The test ink includes at least one chromogen reagent which will undergo a visible color change in the presence of liberated oxygen, and at least one oxygen donor reagent which will liberate oxygen when catalyzed by the presence of hemoglobin. It should be appreciated that the prior art literature lists or catalogs virtually hundreds of chromogens and virtually hundreds of oxygen donors, but the prior art does not necessarily indicate which chromogen-donor pairs or couples are suitable for detecting hemoglobin and which are printable and which may be suitably isolated as to preclude premature interaction as will be hereinafter described. According to the principles of the present invention, we have discovered that a preferred chromogen is 3,3',5,5'-tetramethylbenzidine and a preferred oxygen donor is cumene hydroperoxide (α, α'-dimethylbenzyl hydroperoxide). However, it must be appreciated that it is not satisfactory to merely determine the appropriate quantities of the above two ingredients (or the appropriate quantities of all the other ingredients) necessary to achieve the desired sensitivity, mix them together and deposit them on the test sheet. This does not assure desired sensitivity nor reproducibility of results nor reliability. Thus, it is not merely satisfactory to calculate molarities and provide suitable quantities of chromogen, oxygen donor and any other ingredients for a stoichiometrically balanced reaction. Such a procedure would produce a test composition which would function properly in the laboratory, such as in a test tube, but not necessarily be mechanically printable and not necessarily be functional under normally occurring conditions. In addition, in the system described herein, the chromogen is a solid at room temperature and must be dissolved or solubilized in a solvent which will be inert or inactive relative to the detection of hemoglobin. According to the principles of the present invention, a preferred solvent or surfactant is alkylphenoxypolyethoxyethanol which is marketed as Triton X-100™ by Sigma Chemical Co.

As will be further described, the positive monitor area will contain a substance having peroxidase-like activity, preferably hemoglobin. During the manufacture of the test product and thereafter until the test product is immersed in a toilet bowl, it is important to prevent the positive monitor from catalyzing a reaction between the oxygen donor and the chromogen. For this reason, it may be desirable for the test ink to include a stabilizing agent which prevents the chromogen and oxygen donor from interacting prematurely. A suitable stabilizer is triethanolamine borate, as described in United States Patent No. 4,071,318. However, the stabilizer must be encapsulated and put into solution as part of the preparation of the test ink. To accomplish this objective, the triethanolamine borate is dissolved in a foaming agent such as Stepanol AM™ which is distributed by the Stephan Company of Northfield, Illinois. Furthermore, the preferred foaming agent provides certain additional benefits such as increasing the hydrophilic nature of the test sheet.

We have further discovered that an alternative, equally satisfactory stabilizer is boron phosphate which may also be dissolved in a foaming agent. Lastly we have discovered that within the tolerances of mechanically printing the ink, and using accelerated degradation tests as described in U.S. Patent No. 4,071,318, substantially equivalent results have been obtained without either of the above two stabilizing agents.

In addition, the test ink includes one or more water soluble polymers which encapsulate the test ink and function as a moisture barrier against ambient moisture, e.g., humidity. The moisture barrier should be solid at room temperature, and a preferred polymer is polyvinylpyrrolidone. Preferably a low molecular weight PVP may be used such as PVP 30™. The PVP also tends to render the test area 12 of the sheet 10 hydrophilic, so that the test area 12 wets more readily than the unprinted negative monitor area 18.

As will be readily appreciated by those skilled in the art, the actual concentrations of the ingredients of the composition of the test ink may be varied, which will also result in varying the sensitivity of the test product 10 and the intensity of the color which develops in a positive test. Therefore, although the invention provides a distinct color reaction when as little as 1.5 to 2.0 mg of he-

moglobin per 100 ml of test sample, for a particular application the sensitivity of the kit may be increased to be outside this sensitivity range.

It should be appreciated that the present invention relates to a fecal occult blood test product which is to be utilized by patients within the privacy of their homes. Thus, the results of the use of the specimen test sheet will not be interpreted by medically skilled or medically experienced personnel. For this reason, we have included yet another ingredient in the test ink, namely, a color enhancer such as 6-methoxyquinoline.

In the preparation of the test sheet, the test ink may be deposited on the sheet by various printing techniques such as using an offset press, an ink stamp pad, a flexopress, etc. Various adjustments may be made in the formulation which is hereafter described depending upon the specific printing technique employed. The formulation hereafter described is suitable for use with an offset press or a conventional ink stamp pad.

After the test ink is printed on the entire test sheet except the negative monitor area 18, then a polymer barrier is printed over the entire sheet. Thereafter, a positive monitor ink must be applied to the positive monitor test area 16. A preferred formulation for the positive monitor test ink is a 3% solution of crystalline hemoglobin in Triton X-100™ which is ground on a 3 roller mill and then printed or deposited in the positive monitor area.

A test ink made according to the following example has been evaluated for sensitivity and reproducibility of results. When compared to commercially available fecal occult blood test products which have been approved by the United States Food and Drug Administration, comparable results are achieved by the following formulation.

Example of Test Ink

22.5 grams of Triton X-100™;
1.0 gram 3,3',5,5' tetramethylbenzidine;
6.0 grams of a 10% solution of triethanolamine borate in Stepanol AM, (optional) or 6.0 grams of a 10% solution of boron phosphate in Stepanol AM, (optional);
6.0 grams of Stepanol AM;
2.5 grams of a 5% solution of polyvinylpyrrolidone-30 in Triton X-100™;
10.0 grams of cumene hydroperoxide; and
1.0 gram of 6-methoxyquinoline.

Method of Formulating the Test Ink

We believe that the method of formulating the test ink, i.e., the sequence of adding the ingredients, is important for a successful fecal occult blood test product. First, the chromogen is dissolved in the Triton X-100™ solvent. The stabilizer, if it is to be utilized, is prepared separately, i.e., 6.0 grams of a 10% solution of either boron phosphate or triethanolamine borate in Stepanol AM™. The stabilizer is then added to the chromogen. Then, the additional Stepanol AM is added. This becomes the first portion of the test ink. Then, separately, 2.5 grams of a 5% solution of PVP-30 in Triton X-100™ is prepared. To this solution the oxygen donor is added and to this combination the color enhancer is then added. This becomes the second portion of the test ink. The second portion of the test ink is then added to the first portion of the test ink.

The method of preparing the test sheet will now be explained. The test ink is printed on the entire test sheet excluding the negative monitor test area 18. Then, a polymer barrier is printed on the test ink. The polymer barrier is 10.0 grams of a 5% solution of PVP-30™ in Triton X-100™. Lastly, the positive test ink formulation as described above was printed in the positive monitor test area 16 on top of the polymer barrier. The test sheet prepared according to the aforementioned formulation produced acceptable results comparable to the results obtained with FDA-approved commercially available fecal occult blood test products.

The preferred printing technique heretofore utilized is a dual head offset press. On the first pass of the sheet through the press, the first head prints the test ink and the second head prints the graphics or legends. Then the sheet is sent through the press a second time. During this second pass through the press, the first head prints the polymer barrier and the positive test ink is printed by the second head. It should be appreciated that alternate printing equipment may be used and that the foregoing explanation is merely exemplary.

It should be pointed out that while the above formulation is given as a preferred commercial example, there are, of course, ranges for each of the ingredients. The range for each ingredient may vary by + or - 15% as long as there is an excess of oxygen donor. Although the amounts of ingredients may be changed, we believe that the specific sequence or order of combining the ingredients is of importance. Also, certain ingredients may be substituted for those listed in the above formulation without departing from the scope of the present invention.

A distinction should be made between the formulation of the present invention and the prior art. While the prior film type product (see for example US-A-5081040) uses many of the same ingredients as described herein, there appears to be at least two significant differences. For example, the present invention utilizes about 40-60% of Triton X-100™ as a bridge, or surfactant, or solubilizing agent whereas the prior formula includes only a small percentage of that type of ingredient. In addition, whereas the present invention includes only a small amount of PVP, functioning as a moisture barrier, the PVP is the predominant ingredient, apparently constituting about 75% of the formulation of the prior product. Thus, the mere presence of similar or identical ingredients in the prior art, without regard to their propor-

tions and functions, may inaccurately suggest that the present formulation taken as a whole is a mere trivial variation of prior formulations.

In addition to the foregoing, we believe that the combination of foaming agent, solvent and encapsulator provides superior results insofar as stability and printability.

The use of the fecal occult blood test product of the present invention will now be summarized. After the patient has completed a bowel movement, the test sheet is merely dropped into the toilet bowl. If the chromogen undergoes a color change within the specimen test area, the result of the test is considered positive for fecal occult blood. Conversely, the absence of a color change is considered as a negative result indicating the absence of fecal occult blood. The validity of the test is confirmed by a color change in the positive monitor test area and by the absence of a color change in the negative monitor test area. After the results of the test have been visually observed, the test sheet is disposed of by merely flushing the toilet bowl.

The foregoing is a complete description of a preferred embodiment of the present invention. The invention may be modified as to ingredients and amounts while taking into account the functions of the ingredients. The invention, therefore, should be limited only by the scope of the following claims.

Claims

1. A test sheet for the determination of the presence of a substance having a peroxidase-like activity such as haemoglobin in an aqueous solution, comprising:

   a sheet (10) having a specimen test area (12) and a positive monitor (16) area and a negative monitor area (18), said sheet comprising an inert water insoluble matrix;
   said specimen test area having deposited thereon a first composition comprising at least one oxygen donor reagent dissolved in a solvent comprising a surfactant, at least one chromogen reagent dissolved in a solvent comprising a surfactant, and capable of being oxidized by the oxygen donor in the presence of a substance having the peroxidase-like activity, to provide a visually observable change of colour; and
   said positive monitor test area having deposited thereon said first composition and a second composition comprising a substance having peroxidase-like activity.

2. A test sheet as claimed in claim 1, in which the matrix is a sheet comprising fibres of cellulose.

3. A test sheet as claimed in claim 1 or 2, in which the first composition further includes an inert water soluble matrix, and at least one water soluble polymer functioning as a moisture barrier for said chromogen and said oxygen donor reagents.

4. A test sheet as claimed in claim 3, in which said at least one water soluble polymer includes polyvinylpyrrolidone.

5. A test sheet as claimed in any one of the preceding claims, in which the oxygen donor reagent and the chromogen reagent are deposited on the same surface of the sheet.

6. A test sheet as claimed in any one of the preceding claims, in which the first composition includes a stabilizer.

7. A test sheet as claimed in claim 6, in which the first composition contains between 10% and 14% stabilizer by weight.

8. A test sheet as claimed in any one of the preceding claims, in which the at least one chromogen includes 3,3',5,5'-tetramethylbenzidine.

9. A test sheet as claimed in any one of the preceding claims, in which said first composition includes a moisture barrier.

10. A test sheet as claimed in any one of the preceding claims, in which said second composition includes crystalline haemoglobin.

11. A test sheet as claimed in any one of the preceding claims, in which the oxygen donor agent includes cumene hydroperoxide.

12. A test sheet as claimed in any one of the preceding claims, in which the oxygen donor reagent includes cumene hydroperoxide in an amount from 15% to 25% by weight and the chromogen reagent includes 3,3',5,5'-tetramethylbenzidine in an amount from 1% to 4%.

13. A method of making a test sheet as claimed in any one of the preceding claims which comprises dissolving said at least one chromogen reagent in a solvent comprising a surfactant to form a first portion of said first composition, separately dissolving said at least one oxygen donor in a solvent comprising a surfactant to form a second portion of said first composition, adding said second portion to said first portion to form said first composition, printing said first composition on a sheet (10) in said specimen test area (12) and said positive monitor area (16) but not in said negative monitor area (18) and print-

ing a second composition comprising a substance having peroxidase-like activity in said positive monitor area (16).

14. A method of making a test kit for the determination of the presence of a substance having peroxidase-like activity comprising the steps of:

providing a sheet (10) having a specimen test area (12) and a positive control area (16), said sheet comprising an inert water insoluble matrix;
applying a test ink to said specimen test area and said positive control area (16), said test ink comprising at least one oxygen donor reagent dissolved in a solvent comprising a surfactant, at least one chromogen reagent dissolved in a solvent comprising a surfactant, and capable of being oxidized by the oxygen donor in the presence of a substance having the peroxidase-like activity, to provide a visually observable change of colour; and
applying a positive monitor ink on said test ink in said positive monitor area (16), said positive monitor ink comprising a substance having the peroxidase-like activity.

15. A method as claimed in claim 14, in which the oxygen donor reagent and chromogen reagent are applied on the same surface of the sheet.

16. A method as claimed in claim 14 or 15, in which said sheet further comprises a negative control area characterised by the absence of test ink and positive monitor ink.

17. A method as claimed in claim 14, 15 or 16, in which the applying of said inks involves the step of printing.

18. A method as claimed in any one of claims 14 to 17, in which the at least one chromogen is 3,3',5,5'-tetramethylbenzidine and the step of applying the test ink includes applying a water soluble polymer for encapsulating said chromogen and said oxygen donor for providing a moisture barrier.

Patentansprüche

1. Testblatt zur Bestimmung des Vorliegens einer Substanz mit einer Peroxidase-ähnlichen Aktivität, wie zum Beispiel Hämoglobin in einer wäßrigen Lösung, das folgendes umfaßt:

Ein Blatt (10) mit einem Probentestbereich (12) und einem positiven Kontrollbereich (16) und einem negativen Kontrollbereich (18), wobei genanntes Blatt eine inerte, wasserunlösliche Matrix umfaßt;

genannten Probentestbereich mit einer darauf abgelagerten ersten Zusammensetzung, die folgendes umfaßt: Mindestens ein Sauerstoffdonor-Reagens, das in einem Lösungsmittel aufgelöst ist, das ein Tensid umfaßt, mindestens ein Chromogen-Reagens, das in einem Lösungsmittel aufgelöst ist, das ein Tensid umfaßt und die durch den Sauerstoffdonor bei Vorliegen einer Substanz mit einer Peroxidase-ähnlichen Aktivität oxidiert werden kann, um eine visuell erkennbare Farbänderung bereitzustellen und

genannten positiven Kontrolltestbereich mit darauf abgelagerter genannter erster Zusammensetzung und einer zweiten Zusammensetzung, die eine Substanz mit Peroxidaseähnlicher Aktivität umfaßt.

2. Testblatt nach Anspruch 1, worin die Matrix ein Blatt ist, das Cellulosefasern umfaßt.

3. Testblatt nach Anspruch 1 oder 2, worin die erste Zusammensetzung darüber hinaus eine inerte wasserlösliche Matrix und mindestens ein wasserlösliches Polymer umfaßt, das als eine Feuchtigkeitsschranke für genanntes Chromogen wirkt und genannte Sauerstoffdonor-Reagentien einschließt.

4. Testblatt nach Anspruch 3, worin das genannte, mindestens eine wasserlösliche Polymer Polyvinylpyrrolidon einschließt.

5. Testblatt nach einem der vorangehenden Ansprüche, worin das Sauerstoffdonor-Reagens und das Chromogen-Reagens auf der gleichen Oberfläche des Blattes abgelagert sind.

6. Testblatt nach einem der vorangegangenen Ansprüche, worin die erste Zusammensetzung einen Stabilisator einschließt.

7. Testblatt nach Anspruch 6, worin die erste Zusammensetzung zwischen 10 Gew.-% und 14 Gew.-% Stabilisator enthält.

8. Testblatt nach einem der vorangegangenen Ansprüche, worin mindestens ein Chromogen 3,3', 5,5'-Tetramethylbenzidin einschließt.

9. Testblatt nach einem der vorangegangenen Ansprüche, worin genannte erste Zusammensetzung eine Feuchtigkeitsbarriere einschließt.

10. Testblatt nach einem der vorangegangenen

Ansprüche, worin genannte zweite Zusammensetzung kristallines Hämoglobin einschließt.

11. Testblatt nach einem der vorangegangenen Ansprüche, worin das Sauerstoffdonor-Agens Kumenhydroperoxid einschließt.

12. Testblatt nach einem der vorangegangenen Ansprüche, worin das Sauerstoffdonor-Reagens Kumenhydroperoxid in einer Menge von 15 Gew.-% bis 25 Gew.-% und das Chromogen-Reagens 3,3',5,5'-Tetramethylbenzidin in einer Menge von 1% bis 4% einschließt.

13. Verfahren zur Herstellung eines Testblattes nach einem der vorangegangenen Ansprüche, welches folgendes umfaßt: Auflösen von genanntem, mindestens einem Chromogen-Reagens in einem Lösungsmittel, das ein Tensid zur Bildung eines ersten Anteils von genannter erster Zusammensetzung umfaßt, getrenntes Auflösen von genanntem, mindestens einem Sauerstoffdonor in einem Lösungsmittel, das ein Tensid zur Bildung eines zweiten Anteils von genannter erster Zusammensetzung umfaßt, Zufügen von genanntem zweitem Anteil zu genanntem erstem Anteil zur Bildung von genannter erster Zusammensetzung, Aufdrucken von genannter erster Zusammensetzung auf ein Blatt (10) in genanntem Probentestbereich (12) und genanntem positivem Kontrollbereich (16), aber nicht in genanntem negativem Kontrollbereich (18) und Aufdrucken einer zweiten Zusammensetzung, die eine Substanz mit einer Peroxidase-ähnlichen Aktivität umfaßt, in genanntem positivem Kontrollbereich (16).

14. Verfahren zur Herstellung eines Test-Kits für die Bestimmung des Vorliegens einer Substanz mit Peroxidase-ähnlicher Aktivität, das die folgenden Schritte umfaßt:

Bereitstellen eines Blattes (10) mit einem Probentestbereich (12) und einem positiven Kontrollbereich (16), wobei genanntes Blatt eine inerte wasserunlösliche Matrix umfaßt;

Aufbringen einer Testdruckfarbe auf genannten Probentestbereich und genannten positiven Kontrollbereich (16), wobei die Druckfarbe für den Test folgendes umfaßt: Mindestens ein Sauerstoffdonor-Reagens, das in einem Lösungsmittel aufgelöst ist, das ein Tensid umfaßt, mindestens ein Chromogen-Reagens, das in einem Lösungsmittel aufgelöst ist, das ein Tensid umfaßt und die durch den Sauerstoffdonor bei Vorliegen einer Substanz mit der Peroxidase-ähnlichen Aktivität oxidiert werden kann, um eine visuell erkennbare Farbänderung bereitzustellen und

Aufbringen einer Druckfarbe für die positive Kontrolle auf genannte Druckfarbe für den Test in genanntem positivem Kontrollbereich (16), wobei genannte Druckfarbe für die positive Kontrolle eine Substanz mit der Peroxidase-ähnlichen Aktivität umfaßt.

15. Verfahren nach Anspruch 14, worin das Sauerstoffdonor-Reagens und Chromogen-Reagens auf die gleiche Oberfläche des Blattes aufgebracht werden.

16. Verfahren nach Anspruch 14 oder 15, worin genanntes Blatt darüber hinaus einen negativen Kontrollbereich umfaßt, dadurch gekennzeichnet, daß keine Druckfarbe für den Test und keine Druckfarbe für die positive Kontrolle vorliegt.

17. Verfahren nach Anspruch 14, 15 oder 16, worin das Aufbringen von genannten Druckfarben, den Schritt des Aufdruckens einbezieht.

18. Verfahren nach einem der Ansprüche 14 bis 17, worin das mindestens eine Chromogen 3,3',5,5'-Tetramethylbenzidin ist und der Schritt des Aufbringens der Druckfarbe für den Test das Aufbringen eines wasserlöslichen Polymers zum Einkapseln von genanntem Chromogen und genanntem Sauerstoffdonor zur Bereitstellung einer Feuchtigkeitsbarriere einschließt.

Revendications

1. Feuille de test servant à déterminer la présence d'une substance ayant une activité similaire à celle de la peroxydase, par exemple l'hémoglobine dans une solution aqueuse, comportant:

une feuille (10) ayant une zone test pour le spécimen (12) ainsi qu'une zone de contrôle positif (16) et une zone de contrôle négatif (18), ladite feuille comprenant une matrice inerte insoluble dans l'eau ;
ladite zone test pour le spécimen ayant une première composition deposée là-dessus comprenant au moins un réactif donneur d'oxygène dissous dans un solvant contenant un surfactant, au moins un réactif chromogène dissous dans un solvant contenant un surfactant, et capable d'être oxydée par le donneur d'oxygène en présence d'une substance possédant l'activité similaire à celle de la peroxydase, afin de fournir un changement de couleur qui peut s'observer visuellement ; et
ladite zone test de contrôle positif ayant ladite

première composition et une deuxième composition contenant une substance ayant une activité similaire à celle de la peroxydase.

2. Feuille de test, conformément à la revendication 1, dont la matrice est une feuille comprenant des fibres de cellulose.

3. Feuille de test, conformément à la revendication 1 ou 2, dans laquelle la première composition inclut en outre une matrice inerte soluble dans l'eau et au moins un polymère soluble dans l'eau agissant comme barrière contre l'humidité pour ledit chromogène et lesdits réactifs donneurs d'oxygène.

4. Feuille de test, conformément à la revendication 3, dans laquelle ledit au moins un polymère soluble dans l'eau inclut de la polyvinylpyrrolidone.

5. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle le réactif donneur d'oxygène et le réactif chromogène sont déposés sur la même surface de la feuille.

6. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle la première composition inclut un stabilisateur.

7. Feuille de test, conformément à la revendication 6, dans laquelle la première composition contient entre 10 % et 14 % en poids de stabilisateur.

8. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle le au moins un chromogène contient de la 3,3', 5,5'-tétraméthylebenzidine.

9. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle ladite première composition inclut une barrière contre l'humidité.

10. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle ladite deuxième composition inclut de l'hémoglobine cristalline.

11. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle l'agent donneur d'oxygène inclut de l'hydroperoxyde de cumène.

12. Feuille de test, conformément à l'une quelconque des revendications précédentes, dans laquelle le réactif donneur d'oxygène inclut de l'hydroperoxyde de cumène dans des proportions de 15 % à 25 % en poids et le réactif chromogène inclut de la 3,3', 5,5'-tétraméthylebenzidine dans des proportions comprises entre 1 % et 4 %.

13. Méthode de réalisation d'une feuille de test, conformément à l'une quelconque des revendications précédentes, qui comporte la dissolution dudit au moins un réactif chromogène dans un solvant contenant un surfactant afin de former une première partie de ladite première composition, la dissolution séparée dudit au moins un donneur d'oxygène dans un solvant contenant un surfactant afin de former une deuxième partie de ladite première composition, ajoutant ladite deuxième partie à ladite première partie afin de former ladite première composition, l'impression de ladite première composition sur une feuille (10) dans ladite zone test pour spécimen (12) et dans ladite zone de contrôle positif (16) mais pas dans ladite zone de contrôle négatif (18), et l'impression d'une deuxième composition contenant une substance ayant une activité similaire à celle de la peroxydase dans ladite zone de contrôle positif (16).

14. Méthode de réalisation d'un kit de test pour déterminer la présence d'une substance ayant une activité similaire à celle de la peroxydase comportant les étapes suivantes :

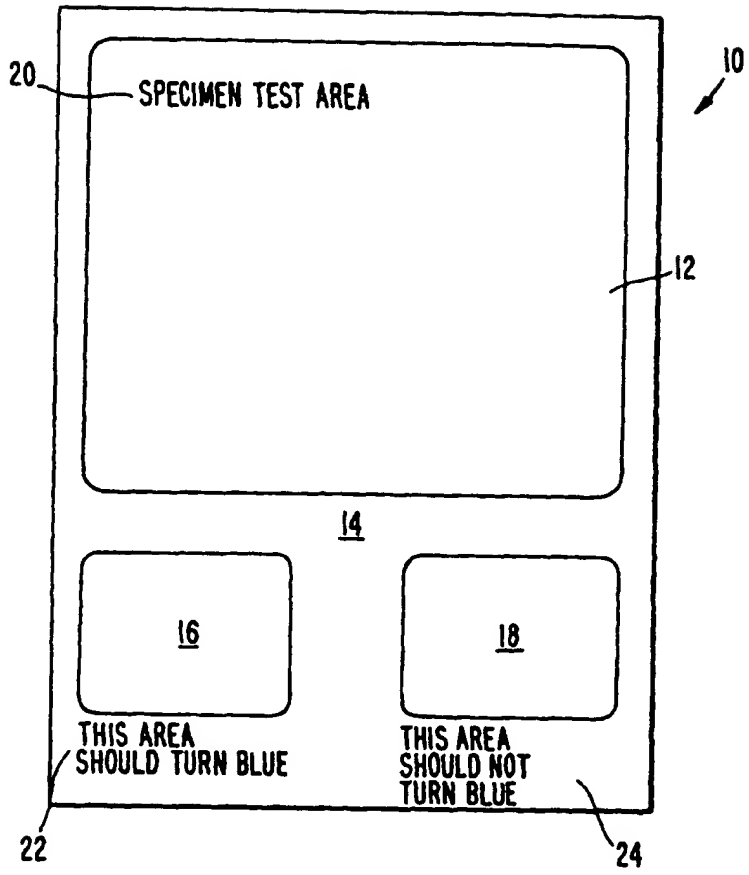
la fourniture d'une feuille (10) ayant une zone test pour spécimen (12) et une zone de contrôle positif (16), ladite feuille ayant une matrice inerte insoluble dans l'eau :
l'application d'une encre test à ladite zone test pour spécimen et à ladite zone de contrôle positif (16), ladite encre test comportant au moins un réactif donneur d'oxygène dissous dans un solvant contenant un surfactant, au moins un réactif chromogène dissous dans un solvant contenant un surfactant, et capable d'être oxydée par le donneur d'oxygène en présence d'une substance ayant une activité similaire à celle de la peroxydase, afin de fournir un changement de couleur qui peut s'observer visuellement ; et
l'application d'une encre de contrôle positif sur ladite encre test dans ladite zone de contrôle positif (16), ladite encre de contrôle positif comportant une substance ayant une activité similaire à celle de la peroxydase.

15. Méthode, conformément à la revendication 14, dans laquelle le réactif donneur d'oxygène et le réactif chromogène sont appliqués sur la même surface de la feuille.

16. Méthode, conformément aux revendications 14 ou 15, dans laquelle ladite feuille comprend également une zone de contrôle négatif caractérisée par l'absence d'encre test et d'encre de contrôle positif.

17. Méthode, conformément aux revendications 14, 15 ou 16, dans laquelle l'application desdites encres comporte l'étape d'impression.

18. Méthode, conformément à l'une quelconque des revendications de 14 à 17, dans laquelle le au moins un chromogène est de la 3,3', 5'5-tétraméthylebenzidine et l'étape d'application d'encre test inclut l'application d'un polymère soluble dans l'eau servant à renfermer ledit chromogène et ledit donneur d'oxygène afin de fournir une barrière contre l'humidité.


20
SPECIMEN TEST AREA
10
12
14
16
18
THIS AREA SHOULD TURN BLUE
THIS AREA SHOULD NOT TURN BLUE
22
24

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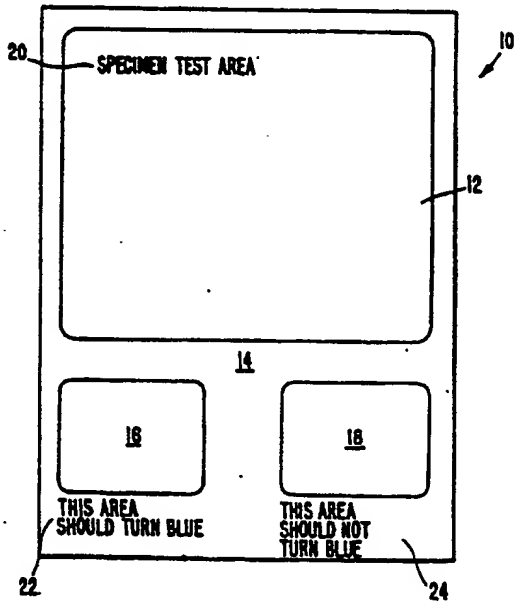
(54) Fecal occult blood test product with positive and negative controls.

(57) A test sheet (10) for the determination of the presence of a substance having peroxidase-like activity in a throw-in-the bowl fecal occult blood test, includes a specimen test area (12) and a positive control area (16). The test area (12) has deposited thereon a test ink having at least one oxygen donor reagent and a chromogen reagent capable of being oxidized by the oxygen donor in the presence of blood or a substance having peroxidase-like activity, to provide a visually observable change of colour. The test ink preferably also includes a water soluble polymer for immobilizing the chromogen and oxygen donor. The positive control area has deposited thereon the test ink and a substance having a peroxidase-like activity.



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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 2918

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X,A	EP-A-0 222 700 (WARNER-LAMBERT COMPANY) * the whole document *	1-5,8,9, 13,14,17, 6,10,12, 15	G 01 N 33/72
X,A	EP-A-0 233 144 (WARNER-LAMBERT COMPANY) * the whole document *	1,2,5,8, 11,13,14, 16,3,4,9, 10,15,1	
X,D,X	GB-A-2 147 416 (HELENA LABORATORIES CORPORATION) * page 6; figures& US-A-4541987 *	1,2,5, 13-15	
D,A	US-A-4 071 318 (TAK WAI LAM) * claims 1, 2 *	1,8	
A	EP-A-0 093 595 (HEMATEC CORPORATION)		
A	EP-A-0 124 215 (WARNER-LAMBERT CORPORATION)		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)
G 01 N A 61 B C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
Berlin	12 February 91	CEDER H O

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